PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 6013-118PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/CA 03/01269	International filing date *(day/month/year)* 20.08.2003	Priority date *(day/month/year)* 20.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant UNIVERSITE LAVAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.03.2004	21.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hermann, P Telephone No. +49 89 2399-7109

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA 03/01269

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-6, 8-23, 25-30, 32, 33, 35, 36, 38, 41, 42, 44	as originally filed
7, 24, 31, 34, 37, 39, 40, 43	received on 12.10.2004 with letter of 12.10.2004

Claims, Numbers

10-29	as originally filed
1-9	received on 12.10.2004 with letter of 12.10.2004

Drawings, Sheets

1/28-28/28	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☒ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-15, 25 and 26 (all in part), 16-20 and 27-29 (in full) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20, 25-29
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-20, 25-29
Industrial applicability (IA)	Yes:	Claims	1-20, 25-29
	No:	Claims	-

2. Citations and explanations

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA 03/01269

see separate sheet

Re Item I

Basis of the opinion

1. Sequence listing pages 1-40 are also included in the basis of this written opinion.

Re Item IV

1. Reference is made to the following documents:

   A: US-B-6 395 481 (RATAIN MARK J ET AL) 28 May 2002 (2002-05-28)
   B: WO-A-02/48400 (ANDO YU-UICHI ;SHIMOKATA KAORU (JP); HASEGAWA YOSHINORI (JP); NAGO) 20 June 2002 (2002-06-20)
   C: Gagne J-F *et al.* - 'Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38)' - September 2002 - Molecular Pharmacology, **62**: 608-617

2. This International Examination Authority considers that there are 3 inventions covered by the claims as follows:

   I: Claims 1-15, 25 and 26 (all in part), 23 and 24 (in full) relates to a method for determining the predisposition of patients to toxicity or lack of efficacy of a biologically active compound comprising detecting polymorphism or haplotic variation in UGT1A1 gene wherein the presence of said polymorphism or haplotic variation is indicative of said predisposition.

   II: Claims 1-15, 25 and 26 (all in part), 21 and 22 (in full) relates to i) a method for determining the predisposition of patients to toxicity or lack of efficacy of a biologically active compound comprising detecting polymorphism or haplotic variation in UGT1A7 gene wherein the presence of said polymorphism or haplotic variation is indicative of said predisposition; and ii) the isolated nucleotide sequence comprising sequences presenting said polymorphism or variation.

   III. Claims 1-15, 25 and 26 (in part), 16-20 and 27-29 (in full) relates to i) a method for

determining the predisposition of patients to toxicity or lack of efficacy of a biologically active compound comprising detecting polymorphism or haplotic variation in UGT1A9 gene wherein the presence of said polymorphism or haplotic variation is indicative of said predisposition; ii) the isolated nucleotide sequence comprising sequences presenting said polymorphism or variation; and iii) the isolated amino acid sequence comprising the translation of said polymorphism or variation.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

Inventions 1-3 relate to methods for the identification of patients presenting a predisposition to toxicity or lack of efficacy of biologically active compound, which method comprise the detection of at least one polymorphism or haplotic variation in the genes encoding UGT1A1, UGT1A7 or UGT1A9, the nucleotide sequences encoding for said variant proteins or the amino acid sequence corresponding too said proteins.

The common concept linking inventions 1-3 is the provision of polymorphism or genetic variation in genes encoding for UDP-glucuronosyltransferase, said polymorphism or variation confering a modification in the drug metabolism due to a modification either of the expression or of the activity of UDP-glucuronosyltransferase.

However, documents A, B and C disclose the identification of polymorphism in the gene encoding UGT1A1 (and also that encoding UGT1A7 in document C) that correlate with altered gene expression/activity and correlated with the sensitivity of patient to biologically active compound - i.e. xenobiotics such as irinotecan (cf. A claims 9-26, 43-63; D2 abstract; B abstract, results and discussion). Therefore in view of A-C said common general concept is not considered inventive.

In the light of the prior art, the 3 different problems to be solved by the present application are the provision of further polymorphisms or genetic variations in genes encoding for UDP-glucuronosyltransferase UGT1A1, UGT1A7 or UGT1A9 respectively, said variations being linked to the predisposition of the individual, to a physiological reaction to a biological active compound. The respective problems are solved by inventions 1-3, relating to specific polymorphisms of UGT1A1, UGT1A7 or UGT1A9

respectively.

Therefore, due to the fact that no other feature could be identified as "special technical feature" bringing a new and inventive contribution over the prior-art, or representing a common inventive link between the subject-matters of inventions 1-3 in the sense of Rule 13.2 PCT, the present application does not meet the requirements of Rule 13.1 PCT with respect to unity of invention, and is considered to consist of the 3 separate inventions given above.

3. Although, the applicant has not paid additional fees, his request to limit the examination to the claimed subject-matter encompassed by the third invention, i.e. the subject-matter claimed in claims 1-15, 25 and 26 (in part), 16-20 and 27-29 (in full), has been taken into consideration and the present given opinion with respect to the provisions of Article 33(1) PCT (i.e. novelty, inventive step and industrial applicability) is given accordingly - i.e. only for claims 1-15, 25 and 26 (in part), 16-20 and 27-29 (in full) insofar as their subject-matter relate to UGT1A9 polymorphism.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: Tukey R. H. and Strassburg C. P. - 'Human UDP-glucuronosyltransferases: metabolism, expression, and disease' - 2000 - *Annual Review of Pharmacology and Toxicology*, **40**: 581-616

D2: Guillemette C. *et al.* - 'Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene' - 2000 - *Pharmacogenetics*, **10**: 629-644

D3: Gagné J.-F. *et al.* - 'Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38)' - *Molecular Pharmacology*, (09-2002), **62**(3), 608-617

2. Novelty (Article 33(2) PCT)

2.1 No prior-art document at hand discloses the subject-matters of claims 1-20 and 25-29, said claims thus meet the requirements of Article 33(2) PCT.

3. Inventive step (Article 33(3) PCT)

3.1 The subject-matters of claims 1 is not considered to involve an inventive step contrary to the requirements of Article 33(3) PCT, the reasons being as follows:

The considerable substrate-specific redundancy in phenolic glucuronidation between many of UGT1A enzyme isoforms including UGT1A1, UGT1A7 and UGT1A9, as well as the encoding gene sequences of said enzymes are very well known in the art and reported in document D1 (cf. D1 p. 594 line 37 - 596 line 42; p. 599 line 22 - p. 606 line 30; Fig. 1-4 and Table 2). Various polymorphisms in the genes encoding UGT1A enzyme isoforms such as UGT1A1 and UGT1A7 have been described and associated to reduced or modified enzymatic activities of said isoform in particular in their ablilities to act as detoxifying agents (see for example the whole disclosure of D2 and D3). Since only one gene locus encodes for all UGT1A enzyme isoforms, the gene encoding UGT1A9 isoform is therefore situated in the same locus as the genes encoding for UGT1A1 or UGT1A7 and it would therefore have been obvious to the skilled person that polymorphisms in the coding sequences of UGT1A9 are also existing, such a polymorphism is in fact already reported in D2 (cf. D2 p. 641 right-hand column 2nd §). Moreover, and as postulated in D2 as well as in D3 the skilled person knowing that such polymorphisms might be linked to a reduced enzymatic activity phenotype (cf. D2 p. 641 right-hand column 2nd §; and D3 abstract and p. 617 left-hand column 1st §), would have been encouraged to test such hypothesis and therefore arrive to the method of claim 1 without the exercise of an inventive skill.

The subject-matter of claim 1 therefore does not involve an inventive step and claim 1 thus does not fulfill the requirements of Article 33(3) PCT.

Moreover, in view of the broad wording with which the purpose of the method of claim 1, and the method itself, are actually formulated (i.e. "determining predisposition to a physiological reaction", "to a biologically active compound","comprising characterising nucleotide sequence of UGT1A9 gene or part thereof of said individual", "wherein the presence of at least one polymorphic variation is indicative of said predisposition"), the claim merely defines the development and validation to be carried out in order to arrive at the invention (i.e. the discovery of mutations and haplotypes in the gene encoding UGT1A9, their potential impact on the enzymatic activity and therefore their potential impact on the response of an individual to a particular yet to be defined physiological reaction). As mentioned above in view of the documents at hand indicating that polymorphisms in the gene encoding UGT1A9 are to be expected and the common knowledge of the skilled person that a polymorphism is potentially linked to a specific phenotype, the method of claim 1 was obvious.

3.2 In view of the prior-art documents at hand, dependent claims 2-20 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step (Article 33(3) PCT) since these additional features appear to be conventional and do not appear to result in any unexpected effect (Article 33(3) PCT).

3.3 For identical reasons as those exposed under point 3.1 above the skilled person without the exercise of inventive skills and by determining the sequences encoding the polymorphisms contained in UGT1A9 isoform using conventional techniques of molecular biology would have arrived to the sequences disclosed in claims 25, 26 or 27-29. Therefore said claims are not considered to meet the requirements of Article 33(3) PCT.

4. Further comments

4.1 The scope of claim 1 is unclear (Article 6 PCT) as it refers to undefined "physiological

reaction", undefined "biologically active compound", and undefined "polymorphism in the gene encoding UGT1A9". This includes polymorphisms that are yet to be discovered and which are not necessarily linked to a change in UGT1A9 activity. For instance, certain polymorphisms disclosed in the present application are shown not to affect UGT1A9 enzymatic activity in a statistically significant manner (cf. table 10 of the present description). Therefore the claimed subject-matter is not properly defined and not properly supported by the description (Article 6 PCT).

Fig. 2 illustrates the entero-hepatic cycle of irinotecan biotransformation;

Fig. 3 illustrates the major role of UGT1A9 in SN-38 glucuronidation;

Fig. 4 illustrates the distribution of SN-38-G formation by human liver samples.

Figs. 5a to 5f illustrate methods for detecting SNPs;

Figs. 6a to 6d illustrate the missence mutations in the human first exons of *UGT1A7* and *UGT1A9* genes;

Fig. 7 illustrates the expression of the UGT1A9 and UGT1A9 proteins in human liver microsomes;

Figs. 8a to 8e illustrate the effect of UGT1A9 promoter polymorphisms on protein expression;

Figs. 9 illustrates the effect of the UGT1A9 (-2152) polymorphic variation on MPA glucuronidation activity;

Fig. 10 illustrates the effect of the UGT1A9 (-1818) polymorphic variation on SN-38 glucuronidation activity;

Figs. 11a to 11d illustrate the effect of the UGT1A9 (-665) polymorphic variation on glucuronidation activity;

Figs 12 illustrates the effect of UGT1A9 (-275) polymorphic variation on MPA glucuronidation activity;

Figs. 13a and 13b illustrate the correlation between the UGT1A9 protein expression and glucuronidation activity;

Figs. 14a to 14d illustrate the relative expression of UGT1A7 and UGT1A9 protein and their relative activities on SN-38;

Figs. 15a to 15c illustrate the glucuronidation rates of the variant UGT1A9 allozymes;

the relative expression of UGT1A9 in each sample and results were monitored by Oneway analyses.

RESULTS

Ten novel polymorphic variations were identified within the *UGT1A9* promoter region, namely a C(-2208)T substitution, a C(-2152)T substitution, a C(-2141)T substitution, a T(-1887)G substitution, a T(-1818)C substitution, a C(-665)T substitution, a T(-440)C substitution, a C(-331)T substitution, a T(-275)A substitution and a, G(-87)A substitution.

UGT1A9 protein expression is highly variable among tested samples, as shown on Fig. 7. Figs. 8a to 8e demonstrate a positive correlations between the presence of mutated nucleic acids in positions -2152 (Fig. 8a), -665 (Fig. 8b), -440 (Fig. 8c), -331 (Fig. 8d) and -275 (Fig. 8e) in the promoter region of the *UGT1A9* gene and the expression of higher level of UGT1A9 proteins.

EXAMPLE IV

Effect of *UGT1A9* polymorphic variations on liver microsomes glucuronidation

One it has been established that polymorphic variations in the promoter region of the UGT1A9 gene can modulated the expression of the UGT1A9, it was interesting to study the impact of these mutation on global glucuronidation by human liver microsomes. Therefore, a correlation study was undergone to determine if correlations could exist between C(-2152)T, T(-1818)C, C(-665)T and T(-275)A variations and SN-38, mycophenolic acid and 4-hydroxyestrone glucuronide formation. Glucuronidation activity was determined for each liver sample in nmoles/mg of proteins/min and further regrouped respective to the genotype of the patient, namely patient carrying a mutation or non-carrying (wild type) patients.

Results

TABLE 9
Frequency of the UGT1A7 alleles

UGT1A7 genotypes[a]	Population (n=317)[b]	Frequency (%)
*1/*1	30	9.46
/*2	57	17.98
***1/*3 *2/*4**	72	22.71
*1/*4	6	1.89
*1/*6	1	0.32
***1/*7 *2/*6**	7	2.21
***1/*8 *3/*6 *4/*7**	1	0.32
*2/*2	39	12.30
*2/*3	55	17.35
*2/*7	3	0.95
***2/*8 *3/*7**	1	0.32
*2/*9	1	0.32
*3/*3	35	11.04
*3/*7	2	0.63
*4/*4	5	1.58
*5/*5	1	0.32
*5/*9	1	0.32
Low activity genotypes[c]	42	13.26
Intermediate activity genotypes[d]	138	43.54

[a] In bold: Genotypes considered to evaluate allelic frequencies.
[b] 167/317 Caucasian ; 150/317 African-American subjects.
[c] With two low activity alleles.
[d] With one low activity allele.

EXAMPLE IV

Relative expression of the UGT1A7 and UGT1A9 variants and SN-38 glucuronidation activities of UGT1A7 and UGT1A9 allozymes

MATERIAL AND METHODS

UGT1A7 and UGT1A9 expression studies

All five novel UGT1A7 variant alleles were generated by PCR site-directed mutagenesis using pcDNA3-vector containing either UGT1A7*1, *2, *3 or *4 variant alleles as the starting construction. Primers having SEQ ID NO: 32, 33, 34 and 35 (Table 7) were used for site-directed mutagenesis. The variant

Recombinant allozyme Western blot analysis.

Semi-quantitative Western blot analyses (Figs. 14a and 14b) showed high levels of immunoreactive UGT protein in all membrane fractions from HEK293 cell lines stably expressing UGTs. An anti-calnexin polyclonal antibody was also used in combination as an internal reference. Significant expression of all UGT1A7 and UGT1A9 alleles was found adequate allowing enzymatic assays to be performed.

EXAMPLE IV

Loss of function variants of the UGT1A7 and UGT1A9 enzymes

MATERIAL AND METHODS

Enzyme assays

Recombinant allozymes were assayed for UGT activity with the two anticancer agents, SN-38 and flavopiridol, as substrates. Microsomal fractions from HEK293 (40 to 60 µg) were added to a reaction mixture (100 µL) containing 50 mM Tris-HCl, pH 7.3, 10 mM $MgCl_2$, 100 µg/mL phosphatidylcholine and 2 mM UDP-glucuronic acid. SN-38 was added in concentrations ranging from 0.1 to 200 µM whereas flavopiridol was used at two concentrations: 5 and 200 µM. Commercially available human liver microsomes (Human Cell Culture Center Inc., Laurel, MD) were incubated in the same conditions for all experiments. Time-course experiments were performed to determine the linearity of the glucuronidation reaction. For the determination of V_{max} and K_m, HEK293 cells stably expressing UGT1A9 enzymes were incubated in the presence of various concentrations of SN-38 ranging from 0.1 to 200 µM and incubated for 30 min as described above whereas UGT1A7 membranes preparations were incubated for 3 hours. All reaction rates were shown to be linear in these conditions. Reactions with SN-38 were stopped by the addition of 200 µL MeOH + 1 % HCl 2N, followed by centrifugation at 14 000 x *g* for 10 minutes. The supernatants were filtered through a 0.22 µm membrane and 100 µL of water was added to the filtrate. For the detection of SN-38 and its glucuronide (SN-38G), 10 µL

In the analysis of UGT1A7 allozymes, the highest SN-38 glucuronidating activity was observed for UGT1A7*1, *2, *6 and *9. Three novel low activity alleles were identified and the *5, *7 and *8 alleles presented 38-76% lower rates of SN-38G formation compared to UGT1A7*1, similar to the range of activity of the *3 and *4 alleles previously identified as low SN-38 glucuronidating activity alleles (Gagne *et al.*, 2002, *Mol. Pharmacol.* 62:608-617).

TABLE 10

Kinetic parameters for SN-38 glucuronidation by human UGT1A9 allozymes

UGT1A9 allozymes	**Apparent** K_m (µM)	**V_{max}** (pmol/min/mg protein)	**Catalytic efficiencies** V_{max}/K_m (µL/h/mg)
UGT1A9*1	3.02 ± 0.51	316.34 ± 52.03	105
UGT1A9*2	5.15 ± 1.81	324.38 ± 95.09	63
UGT1A9*3	3.21 ± 0.95	11.89 ± 2.61 *	4

The values of apparent K_m and V_{max} for the formation of SN-38 glucuronide were determined using microsomal preparations from UGT1A9-HEK293 cells. Values were expressed as the mean ± SD of at least three independent experiments performed in duplicate from Lineweaver-Burk plots. * $p < 0.002$ compared to UGT1A9*1.

EXAMPLE V

Immunofluorescence localization of UGT1A9*1, UGT1A9*2 and UGT1A9*3 proteins.

MATERIAL AND METHODS

Immunofluorescence visualization

One cSNP found in the UGT1A9 first exon was located in the signal peptide, thus immunofluorescence experiments were designed to localize the expressed protein within the cells. Stable HEK293 cells expressing human UGT1A9*1, UGT1A9*2 and UGT1A9*3 and also with cells transfected with pcDNA3 vector

autofluorescence (Figs. 16a, b and c). UGT1A9*1, UGT1A9*2 and UGT1A9*3 proteins were localized in the cytoplasm and the perinuclear zone as well as in the endoplasmic reticulum (Figs. 16d, g and j).

EXAMPLE VI

Effect of UGT1A1 TATA box variations on UGT1A1 protein expression and glucuronidation activity

Although a correlative association between TATA box polymorphic variation is reported in prior art, the UGT1A7 and UGT1A9 interindividual variations of the present invention remained unknown at this time and their effect on SN-38 glucuronidation therefore remained unconsidered. In an attempt to decipher the particular function of every participating isoform in SN-38-G formation, we were interested to determine whether or not a correlative association could be made between the number of TA repeat in the TATA box of UGT1A1 promoter region and UGT1A1 protein expression even thought novel polymorphic variations were taken into account. As shown in Fig. 17, the presence of TA_6 genotype on both alleles is associated with a higher protein expression while the presence of a TA_7 repeat on only one allele is sufficient to decrease UGT1A1 protein expression. The lowest protein expression level is observed with TA_7 homozygous patients. As shown in Figs. 17b and 17c, the correlative association is also observed between glucuronidaton of the probe substrate estradiol and the number of TA repeats. A similar correlative association is found with SN-38.

As UGT1A1 is considered as a major SN-38 glucuronidation enzyme, we attempted to determine if an association between the expression of this protein and glucuronide formation could exist. As shown in Fig. 18a, there is a positive correlations between glucuronidation of SN-38 and protein level of UGT1A1. To ascertain that the enhancement of glucuronidation observed with this substrate is not attributable to a residual activity of other UGT isoforms, this experiment was reconducted using probe substrates for UGT1A1, namely estradiol,. As seen in fig. 18b a positive correlation exists between UGT1A1 protein level and estradiol-3-G formation. Since estradiol is an endogenously produced

compound and formation of estradiol-3-G is exclusively mediated by UGT1A1, these results demonstrate that a biochemical analysis of serum estradiol-3-G could be properly used to monitor a higher or lower UGT1A1 expression in a patient and therefore, be used as an indicator for determining a predisposition to a physiological reaction to a xenobiotic or an endogenous compound. Finally, Fig. 19 shows the predictive value of the haplotype determination of UGT1A9 and UGT1A1. This haplotype determination includes the genotyping of the *UGT1A9* promoter region and the determination of the number of TA repeats in the TATA box of the *UGT1A1* promoter, which is a more accurate indicator of SN-38 glucuronidation level than the determination of the TA repeats in the TATA box of the *UGT1A1* promoter alone.

EXAMPLE VI

Haplotyping the UGT1A genes

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Differences in kinetic parameters between *UGT* allelic variants were evaluated for statistical significance by paired Student's *t* test. All tests were two-sided. The haplotype frequencies will be estimated using the PHASE 1.0.1 software and Hardy-Weinberg equilibrium and linkage disequilibrium analyses will be performed using ARLEQUIN 2.0™ software.

RESULTS

Analysis of the haplotypic structure of the *UGT1* gene in subjects with UGT1A9*1 or UGT1A9*3 alleles.

Haplotypes of the *UGT1A* gene were analyzed in subjects with the UGT1A9*1/*3 low SN-38 glucuronidation activity genotype.

TABLE 15

Functional UGT1A1, UGT1A7 and UGT1A9 SNPs frenquence in the French-Canadian population.

	UGT1A9 Codon 33		UGT1A7 Codon 208		UGT1A1 TATA box	
Wild-type allele	T	0,98	T	0,62	6	0,67
Mutant allele	C	0,02	C	0,38	7	0,33

EXAMPLE VII

Multiple protein sequence alignment of UGT1A proteins at selected positions

UGT1A7*1, UGT1A9*1 and their genetic variant proteins UGT1A7 (a) and UGT1A9 (b) are aligned with close members of the UGT1A subfamily and the rat UGT1A7 isoenzyme. The varying amino acid positions are indicated with bold characters.

DISCUSSION

After resequencing the first exons of *UGT1A7* and *UGT1A9* genes, 4 polymorphic sites in the targeted regions were identified. Two polymorphic UGT1A9 variants were discovered, UGT1A9*2 $C^{3}Y$ and UGT1A9*3 $M^{33}T$. In addition, the presence of two novel nonsynonymous UGT1A7 SNPs, $G^{115}S$ and $E^{139}D$, combined with previously described missense polymorphisms at codons 129/131 and 208, generated five additional UGT1A7 alleles (*5 through *9). Based on the *in vitro* functional genomic assays, the UGT1A7*3, *4, *5, *8 and *9 alleles and the UGT1A9*3 allele were all identified as low SN-38 glucuronidating alleles. Results demonstrate that the coinheritance of UGT1A1, UGT1A7 variants and especially the loss of function UGT1A9 polymorphism determine individual's susceptibility to irinotecan-induced toxicity. Thus, findings lay emphasis on the necessity to analyze combination of UGT1A1, UGT1A7 and UGT1A9 polymorphisms (haplotypes) rather than looking for a single